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The evolution of vegetative desiccation tolerance in land plants

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Abstract

Vegetative desiccation tolerance is a widespread but uncommon occurrence in the plant kingdom generally. The majority of vegetative desiccation-tolerant plants are found in the less complex clades that constitute the algae, lichens and bryophytes. However, within the larger and more complex groups of vascular land plants there are some 60 to 70 species of ferns and fern allies, and approximately 60 species of angiosperms that exhibit some degree of vegetative desiccation tolerance. In this report we analyze the evidence for the differing mechanisms of desiccation tolerance in different plants, including differences in cellular protection and cellular repair, and couple this evidence with a phylogenetic framework to generate a working hypothesis as to the evolution of desiccation tolerance in land plants. We hypothesize that the initial evolution of vegetative desiccation tolerance was a crucial step in the colonization of the land by primitive plants from an origin in fresh water. The primitive mechanism of tolerance probably involved constitutive cellular protection coupled with active cellular repair, similar to that described for modern-day desiccation-tolerant bryophytes. As plant species evolved, vegetative desiccation tolerance was lost as increased growth rates, structural and morphological complexity, and mechanisms that conserve water within the plant and maintain efficient carbon fixation were selected for. Genes that had evolved for cellular protection and repair were, in all likelihood, recruited for different but related processes such as response to water stress and the desiccation tolerance of reproductive propagules. We thus hypothesize that the mechanism of desiccation tolerance exhibited in seeds, a developmentally induced cellular protection system, evolved from the primitive form of vegetative desiccation tolerance. Once established in seeds, this system became available for induction in vegetative tissues by environmental cues related to drying. The more recent, modified vegetative desiccation tolerance mechanism in angiosperms evolved from that programmed into seed development as species spread into very arid environments. Most recently, certain desiccation-tolerant monocots evolved the strategy of poikilochlorophylly to survive and compete in marginal habitats with variability in water availability.

Introduction

Desiccation tolerance, the ability to recover from the almost complete loss (80–90%) of protoplasmic water, is a phenomenon common in the reproductive structures of green plants, pollen, spores and seeds. However, the ability to survive desiccation in the vegetative stage is a widespread but uncommon occurrence in the plant kingdom generally (Bewley & Krochko 1982; Oliver & Bewley 1997). The majority of vegetative desiccation-tolerant plants are found in the less complex clades that constitute the algae, lichens and bryophytes. In a survey of the literature, Bewley & Krochko (1982) determined that within the larger and more complex groups of vascular land plants there are some 60 to 70 species of ferns and fern allies, and approximately 60 species of angiosperms that exhibit some degree of vegetative desiccation tolerance (Figures 1 and 2). The only major class of vascular plants that does not have a species that has desiccation-tolerant vegetative tissues is the gymnosperms (a taxonomic group consisting of the phylogenetically distinct cycads, conifers, and gnetophytes); Bewley & Krochko (1982) postulate that there may be a mini-

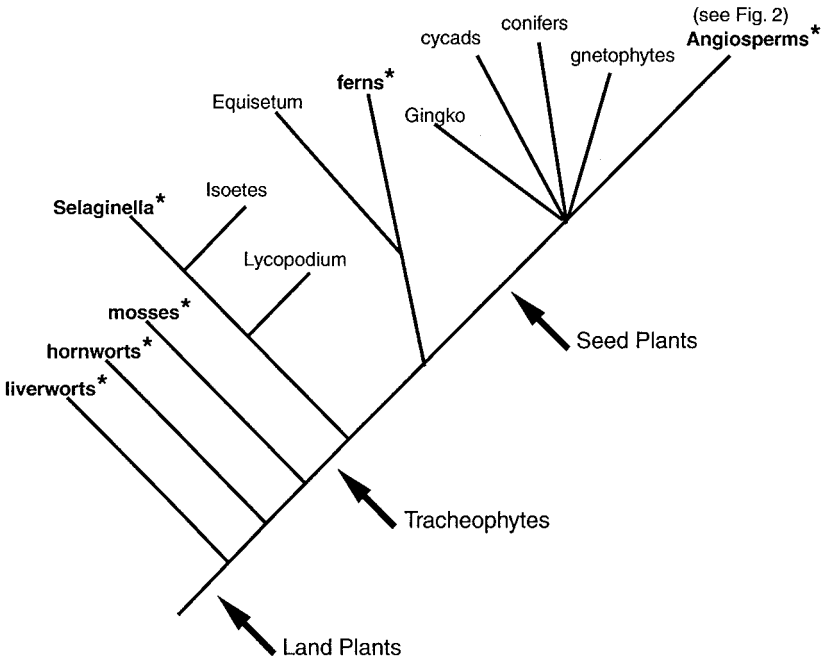

Figure 1. A phylogeny of the major groups of land plants, based on a consensus of several recent synthetic studies (Mishler & Churchill 1985; Crane 1990; Donoghue 1994; Mishler et al. 1994; Kenrick & Crane 1997), and information from workshops of the Green Plant Phylogeny Research Coordination Group. Names in bold and with an asterisk indicate clades with some known desiccation-tolerant members. Parsimony would suggest that while desiccation tolerance was primitive for the land plants, it was then lost early in the evolution of the tracheophytes, followed by at least one independent evolution (or re-evolution) of desiccation tolerance in *Selaginella*, in the ferns, and in the Angiosperms (see Figure 2 for a hypothesis of phylogenetic relationships within the Angiosperms).

mum size limitation for desiccation tolerance, which members of this group exceed.

Recent synthetic phylogenetic analyses (summarized in Figures 1 and 2) suggest that vegetative desiccation tolerance was primitively present in the bryophytes (the basal-most living clades of land plants), but was then lost in the evolution of tracheophytes. We postulate that the initial evolution of vegetative desiccation tolerance was a crucial step required for the colonization of the land by primitive plants from a fresh water origin (Mishler & Churchill 1985), but that tolerance came at a cost, since metabolic rates in tolerant plants are low compared to those in desiccation-sensitive plants. Thus, the loss of tolerance might have been favored along with the internalization of water relationships that happened as the vascular plants became more complex. However, at least one independent evolution (or re-evolution) of desiccation tolerance occurred in *Selaginella* and again in the ferns. Within the angiosperms, at least eight independent cases of evolution (or re-evolution) of desiccation tolerance occurred. The natural rates of desiccation, rehydration and responses to dehydration are different in each of these lineages. This phylogenetic evidence, combined with what we can deduce of the mechanisms by which plants achieve vegetative desiccation tolerance, leads to a hypothesis as to the nature and progression of the evolution of this trait. It is this hypothesis which serves as the focus of this discourse.

Summarizing earlier studies, Bewley (1979) concluded that there are three criteria which a plant or plant structure must meet in order to survive severe loss of protoplastic water. It must: (1) limit the damage incurred to a repairable level, (2) maintain its physiological integrity in the dried state (perhaps for extended periods of time), and (3) mobilize repair mechanisms upon rehydration which effect restitution of damage suffered during desiccation (and upon the inrush of water back into the cells). Bewley & Oliver (1992) interpret these criteria to suggest that desiccation tolerance can be achieved either by mechanisms that are based on the protection of cellular integrity or mechanisms that are based on the repair of desiccation- (or rehydration-) induced cellular damage. As we will explain in detail, more recent studies

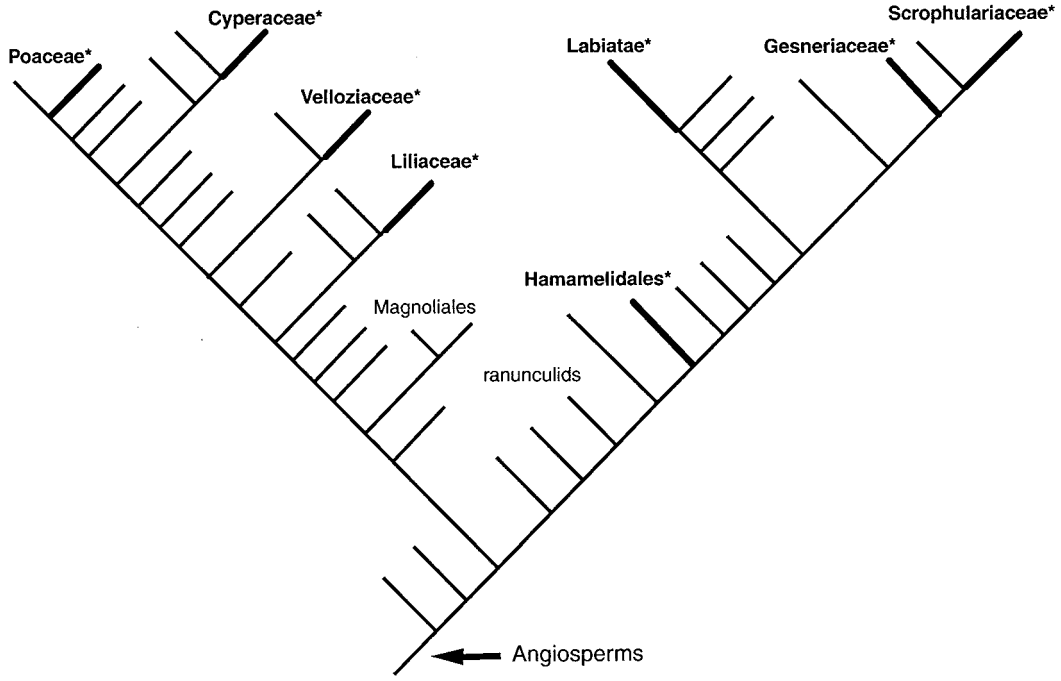

Figure 2. A diagrammatic phylogeny of the major groups of Angiosperms, based on cladistic analysis of the *rbc*L gene by Chase et al. (1993), summary cladograms in Judd et al. (1999), and information from workshops of the Green Plant Phylogeny Research Coordination Group. A few selected taxa are shown for orientation. Branches without names represent (in some cases large) clades with no known desiccation-tolerant members. Names in bold and with an asterisk indicate all clades with some known desiccation-tolerant members. Parsimony would suggest at least one independent evolution (or re-evolution) of desiccation tolerance in each clade (i.e., at least eight times in the Angiosperms).

have corroborated this interpretation and suggested that vegetative desiccation-tolerant plants can be classified into two classes, according to whether their particular mechanism of tolerance places an emphasis upon cellular protection or cellular repair (Oliver & Bewley 1997). Vegetative desiccation-tolerant plants can also be classified into two groups according to their sensitivity to rapid water loss. Some vegetative desiccation-tolerant plants can withstand desiccation only if it occurs slowly, taking anywhere from 12 h to several days to reach the air-dried state. Other plants can survive water loss even if the air-dried state is achieved within an hour. From all that we know so far (see below), it also appears that those vegetative desiccation-tolerant plants that can survive desiccation even if water loss is rapid utilize a mechanism for tolerance that relies heavily on cellular repair (although cellular protective mechanisms also play a role). In contrast, plants that can survive desiccation only if water loss is gradual rely predominantly upon cellular protection as a mechanism for tolerance (for full review, see Oliver & Bewley 1997). It is how these classes relate phylogenetically that is important in understanding the evolution of vegetative desiccation tolerance.

Vegetative desiccation-tolerant plants can be separated into different classes by a third criterion, which also has importance from an evolutionary standpoint. Some vegetative desiccation-tolerant plants dismantle their photosynthetic apparatus and lose their chlorophyll content during desiccation (Tuba et al. 1998). These plants have been termed poikilochlorophyllous (Hambler 1961; Gaff 1977, 1989; Bewley 1979). Desiccation-tolerant plants that retain their photosynthetic apparatus in the dried state are termed homoiochlorophyllous. Plants that require a slow rate of water loss to survive desiccation can be either poikilochlorophyllous or homoiochlorophyllous. All plants that can withstand rapid water loss are homoiochlorophyllous. For a comparison of homoiochlrorophyllous and poikilochlorophyllous plants, see the review by Tuba et al. (1998).

In the following narrative, we will present the evidence that allows us to classify vegetative desiccation-tolerant plants in these ways and explain how these classifications allow us to set up a hypothesis for how desiccation tolerance has evolved.

Plants that survive rapid desiccation

All vegetative desiccation-tolerant plants studied to date that are capable of surviving desiccation regardless of the rate of water loss are of the less complex groups of plants; algae, bryophytes or lichens (Bewley & Krochko 1982; Oliver & Bewley 1997). The internal water content of these plants rapidly equilibrates to the water potential of the environment as they possess very little in the way of water-retaining morphological or physiological characteristics (for lichens see Beckett 1995; for bryophytes see Proctor et al. 1998). As a result of this, many lichens, algae and desert bryophytes experience drying rates that are extreme. By a phylogenetic parsimony argument, the mechanism of tolerance exhibited by these basal clades is the most primitive form of tolerance to desiccation. As the complexity of land plants increased, the ability to survive rapid desiccation was then lost. What do we understand about the mechanism by which these plants survive protoplasmic water loss?

The majority of the studies on this question involve the desiccation-tolerant moss, *Tortula ruralis* (Hedw.) Gaert., Meyer, and Scherb. Freeze fracture studies of dried *T. ruralis* cells (both rapidly and slowly dried) clearly demonstrate that cellular integrity is maintained during drying (Platt et al. 1994). Plasmamembranes and internal membranes and structures are undamaged by the loss of water in *T. ruralis*. However, upon rehydration gametophytic cells undergo substantive and universal disruption of cellular integrity including breaches to all membrane systems (see Oliver & Bewley 1984a for review). Internal organelles swell and distort and their internal membrane systems become dispersed. Nevertheless, the cells do not die, as do cells of sensitive species, but return to a normal appearance within 12 to 24 h. The amount of cellular disruption that occurs during rehydration clearly depends upon the rate at which water was lost during desiccation. Chloroplasts of *T. ruralis* dried to air dryness over 4 to 6 h (a natural rate, M. J. Oliver, unpublished observations), are swollen when rehydrated but retain more of their normal internal structure and exhibit fewer clefts in their membranes than do the chloroplasts in rehydrated cells of gametophytes dried within an hour (Tucker et al. 1975; Tuba unpublished observations). The greater retention of chloroplast structure allows slow dried *T. ruralis* to effect a more rapid recovery of photosynthesis achieving a positive carbon balance within 20 min following rehydration (Bewley 1979; Tuba et al. 1996). The time required for full photosynthetic recovery upon rehydration, however, varies considerably among species depending on their degree of desiccation tolerance (Proctor et al. 1998). Chloroplast swelling and lamellar disruption upon rehydration have also been reported for other mosses such as *Pleurozium schreberi* (Willd.) Mitt (Noailles 1978), and *Barbula torquata* Tayl. and *Triquetrella papillata* (Mook. F. & Wils.) Broth (Moore et al. 1982). Electrolyte leakage upon rehydration, a measure of membrane damage, is also affected by the speed at which desiccation occurs. After slow drying, leakage in moss is less than half as great as after rapid desiccation and similar to leakage of hydrated controls, indicating minimal membrane damage (Bewley & Krochko 1982). These observational studies lead to the hypothesis that desiccation-tolerant bryophytes survive desiccation by a combination of protective measures that allow for the maintenance of cellular order during drying and a repair-based strategy to recover from the damage incurred upon rehydration.

Desiccation of gametophytic tissues of *T. ruralis* results in a rapid decline in protein synthesis, as in all desiccation-tolerant and intolerant mosses tested so far (see Bewley & Oliver 1992; Oliver & Bewley 1997, for reviews). In *T. ruralis* this loss of protein synthetic capacity is manifested by a loss of polysomes resulting from the run-off of ribosomes from mRNAs, concomitant with their failure to reinitiate protein synthesis (see Bewley 1979; Bewley & Oliver 1992, for reviews). The rapid loss of polysomes during drying and the apparent sensitivity of the initiation step of protein synthesis to protoplasmic drying lead to the conclusion that the protection component of the mechanism of tolerance for these plants does not involve the synthesis of proteins induced by the onset of a water deficit. This is borne out by the observation that no new mRNAs are recruited into the protein synthetic complex even if the rate of water loss is slow (Oliver 1991, 1996). The fact that the moss survives rapid desiccation (even when desiccation is achieved in a few minutes in a lyophilizer), also indicates that an inducible protection mechanism is not necessary for survival.

This has led to the suggestion that there is a constitutive protection component to the mechanism of tolerance in *T. ruralis* and similar species. This hypothesis is strengthened by observations concerning the behavior of two cellular components that are purported to offer protection from damage during desiccation; viz., sucrose (see Crowe et al. 1992 for review) and dehydrins (see Close et al. 1993; Dure 1993 for reviews).

Sucrose is the only free sugar available for cellular protection in desiccation-tolerant mosses, including *Tortula ruraliformis* (Besch.) Grout and *T. ruralis* (Bewley et al. 1978; Smirnoff 1992). The amount of this sugar in gametophytic cells of *T. ruralis* is approximately 10% of dry mass, which is sufficient to offer membrane protection during drying, at least in vitro (Straus & Hauser 1986). Moreover, neither drying nor rehydration in the dark or light results in a change in sucrose concentration, suggesting it is important for cells to maintain sufficient amounts of this sugar (Bewley et al. 1978). The lack of an increase in soluble sugars during drying appears to be a common feature of desiccation-tolerant mosses (Smirnoff 1992). The existence of dehydrins in desiccation-tolerant vegetative tissues of desiccation-tolerant bryophytes has only recently been reported. Western blots using purified antibodies raised against the common carboxy-terminus of corn seedling dehydrins (Close et al. 1993) show that *T. ruralis* produces two major dehydrins (80–90 kD and 35 kD). These are present in the hydrated state and do not appear to increase during rapid or slow drying (Bewley et al. 1993). A similar result was obtained with the desiccation-tolerant moss *Thuidium delicatulum* (Hedw.) Mitt. (T. L. Reynolds, M. J. Oliver & J. D. Bewley, unpublished data).

Although extensive induction of recovery mechanisms appears to be precluded during drying of gametophytic tissue of fully desiccation-tolerant bryophytes, there does appear to be some capacity to prepare for recovery upon rehydration. Using cDNA clones corresponding to *T. ruralis* transcripts that are preferentially translated during rehydration (Scott & Oliver 1994), it was determined that several 'recovery' transcripts accumulate during slow drying (Oliver & Wood 1997; Wood & Oliver 1999). These transcripts do not accumulate during rapid desiccation, nor is their accumulation during slow drying associated with an increase in endogenous ABA accumulation. ABA is undetectable in this moss (Bewley et al. 1993; M. J. Oliver, unpubl data), and *T. ruralis* does not synthesize specific proteins in response to applied ABA. Recent studies clearly demonstrate that these transcripts are sequestered in the dried gametophytes in mRNP particles (Wood & Oliver 1999). The implication from this work is that the sequestration of mRNAs required for recovery hastens the repair of damage induced by desiccation or rehydration and thus minimizes the time needed to restart growth upon rehydration. These findings may also explain the ability of *T. ruralis* to 'harden' during recurring desiccation events in the absence of inducible dehydrin or sugar responses (Schonbeck & Bewley 1981a,b).

The repair aspect of the mechanism of desiccation tolerance in these plants, although demonstrated to be a major component of tolerance, is difficult to detail and characterize. Most work has centered on the proteins whose synthesis is induced immediately upon rehydration of desiccated gametophytic tissue. Early work (see Bewley 1979, for review) established the ability of *T. ruralis* and other mosses to rapidly recover synthetic metabolism when rehydrated. The speed of this recovery was dependent upon the rate of prior desiccation; the faster the rate of desiccation, the slower the recovery. In addition, although the pattern of protein synthesis in the first two hours of rehydration of *T. ruralis* is distinctly different from that of hydrated controls, novel transcripts were not made in response to desiccation (Oliver 1991; Oliver & Bewley 1984b). Hence it was suggested that *T. ruralis* responds to desiccation by an alteration in protein synthesis upon rehydration that is in large measure the result of a change in translational control. Changes in transcriptional activity were observed for nearly all transcripts studied (Scott & Oliver 1994) but did not result in a qualitative change in the transcript population during desiccation or rehydration. It thus appears that *T. ruralis* relies more upon the activation of pre-existing repair mechanisms for desiccation tolerance than it does on either pre-established or activated protection systems.

In a detailed study of the changes in protein synthesis initiated by rehydration in *T. ruralis*, Oliver (1991) demonstrated that during the first two hours of hydration the synthesis of 25 proteins is terminated, or substantially decreased, and the synthesis of 74 proteins is initiated, or substantially increased. Controls over changes in synthesis of these two groups of proteins, the former termed hydrins and the latter rehydrins, are not mechanistically linked. It takes a certain amount of prior water loss to fully activate the synthesis of rehydrins upon rehydration. This may in turn indicate that there is also a mechanism by which the amount of water loss is 'sensed' and 'translated' into a protein synthetic response upon rehydration. Such a scenario was also proposed for the novel pattern of protein synthesis associated with the drying of *Sporobolus stapfianus* Gandoger (Kuang et al. 1995). Perhaps this is a strategy which has evolved to link the amount of energy expended in repair to the amount of damage potentiated by differing extents of drying.

Eighteen rehydrin cDNAs, isolated by Scott & Oliver (1994), have been sequenced (Oliver et al. 1997; Wood et al. 1999). Only three exhibit significant sequence homology to known genes in the Genbank databases. Tr155 has a strong sequence similarity to an alkyl hydroperoxidase linked to seed dormancy in barley (Aalen et al. 1994) and *Arabidopsis* embryos (Haslekas et al. 1998), and in rehydrated but dormant *Bromus secalinas* L. seeds (Goldmark et al. 1992). Tr213 exhibits a high degree of similarity to polyubiquitins from several plant sources. The finding that polyubiquitin is a rehydrin is indicative of an increased need for protein turnover during recovery from desiccation. Tr 288 has a dehydrin-like K box sequence at its carboxy terminus of the predicted protein but little similarity to known dehydrins other than similarities in its predicted secondary structure. Nevertheless, that this dehydrin-like protein is synthesized in response to rehydration and not desiccation is intriguing and may indicate that these proteins have a role in damage repair as well as protection from damage.

In addition to our rehydrin analyses, we have also established a small Expressed Sequence Tag (EST) database (the sequences of all transcripts present during the rehydration response) for *T. ruralis* (Wood et al. 1999) from a cDNA library constructed from slow-dried gametophyte polysomal RNA (in an attempt to target sequences sequestered in mRNPs, see above). Of 152 ESTs that were generated and partially sequenced, only 30% showed significant homology to previously identified nucleic acid and/or polypeptide sequences. Interestingly, several ESTs showed significant similarity to unidentified desiccation tolerance genes isolated from the desiccation-tolerant angiosperm *Craterostigma plantagineum* Hochst. (see below). As for the *Craterostigma* EST study (Bockel et al. 1998), the similarity analysis of *T. ruralis* ESTs revealed genes whose identity indicated the involvement of several cellular processes in the response to desiccation (Wood et al. 1999). Further studies are needed before their importance in cellular repair and desiccation tolerance can be elucidated. The possible homology of genes that were involved in the original desiccation tolerance syndrome in bryophytes to those involved in the various re-evolutions of tolerance in the tracheophytes is intriguing and worthy of further study through comparative genomics.

From an ecological standpoint the desiccation-tolerant plants of the less complex clades have the advantage not only of surviving rapid desiccation but also of rapid recovery. Such plants can survive desert-like conditions by being able to utilize small amounts of water and short periods of water availability. Desiccation-tolerant bryophytes and lichens maintain a small cell volume compared to the more complex tracheophytes, which apparently adds to this capability by reducing the physical stresses involved in desiccation and rehydration. This pattern of adaptation is not only seen in desert habitats (Lange et al. 1970) but also in more temperate climes (Csintalan et al. 1998), where it enables the plants to maintain a positive carbon balance during the dry summer season by taking advantage of the short-lived morning dews.

Plants that survive desiccation only if water loss is gradual

The vast majority of vegetative desiccation-tolerant plants that survive desiccation only if the rate of water loss is slow, from several hours to days, belong to the more complex land plant groups, from ferns to angiosperms, and have evolved most recently from non-tolerant ancestors. It is for this reason that this class of plants has been designated as modified desiccation-tolerant by Oliver & Bewley (1997). This term is meant strictly to indicate that this type of tolerance has not evolved directly from the more primitive form of tolerance.

At present we know about the mechanisms of modified desiccation tolerance mostly from the study of two species, *Craterostigma plantagineum*, a southern African dicot; and *Sporobolus stapfianus* Gandoger, an African desert grass (for reviews see Bewley & Oliver 1992; Ingram & Bartels 1996; Oliver et al. 1997; Oliver & Bewley 1997). The mechanisms of tolerance of the pteridophytes *Selaginella lepidophylla* Hook. & Grev., *Polypodium virginianum* L., *P. polypodioides* L. and *Ceterach officinarum Lam. et DC.* have been investigated in a more limited way (see Oliver & Bewley 1997; Muslin & Homann 1992; Schwab et al. 1989), and, some exciting work on the desiccation-tolerant poikilochlorophyllous monocot *Xerophyta scabrida* (Pax) Duir & Schinz has extended the overall picture (Tuba et al. 1994 and 1996), as we will discuss. Moreover, other plants are under study, and our knowledge in this area will advance rapidly. The prevailing evidence from these systems supports the idea that the modified desiccation-tolerant angiosperms appear to rely almost totally on a very effective cellular protection-based mechanism that is induced during drying and requires a certain amount

of time to become established in the leaf tissues. This is, in all likelihood, why such plants cannot survive rapid water loss.

Much of what is known about cellular protection systems for desiccation tolerance in plant cells has come from investigations into the events that surround the programmed maturation drying event during seed development. This, of course, leads to several interesting questions concerning the evolution of desiccation tolerance, since it is clear from the phylogenetic trees that the evolution of desiccation-tolerant vegetative cells preceded the evolution of desiccation-tolerant seeds: (1) Did the mechanism of desiccation tolerance exhibited in seeds evolve from the primitive mechanism seen in the basal clades or independently of it? (2) If tolerance of seeds evolved independently, were some of the original desiccation tolerance genes 'reused' in the acquisition of desiccation tolerance by seeds? (3) Did the mechanisms of vegetative tolerance seen in the modified desiccation-tolerant species derive from the mechanism exhibited by seeds? The answers to these questions have important connotations, because such information could help us to predict the presence of genes in non-tolerant crop species that may have been recruited into different cellular processes. To address these questions we must first briefly outline the pertinent features of the mechanism of seed desiccation tolerance.

The ability of seeds to withstand desiccation is acquired during their development. This acquisition is usually substantially earlier than the drying event itself. Seeds of some species can withstand premature desiccation, from well before to the mid-point of their development (Harlan & Pope 1992; Wellington 1956; Kermode & Bewley 1985). Even somatic embryos can be induced to survive desiccation, generally following exposure to ABA (Janick et al. 1993; Senaratna et al. 1990), despite the fact that the vegetative tissues from which they are derived are intolerant of drying. In all cases the germinating seed itself retains its desiccation tolerance, which is lost at the time of radicle emergence when germination is complete.

Several metabolic changes occur within seeds just prior to, or during, maturation drying. These include the synthesis of sugars and proteins, which have long been postulated to form the basis of a series of overlapping protective mechanisms which limit damage to cellular constituents (Bewley 1979; LePrince et al. 1993; Oliver & Bewley 1997). Although a complete picture of these protective mechanisms is far from realization, two likely components have been identified: (1) accumulation of ABA-induced protective proteins (Lane 1991); and (2) accumulation of non-reducing sugars capable of stabilizing membrane structure in the desiccated state (Crowe et al. 1988) and limiting free radical damage (LePrince et al. 1990).

A highly abundant set of hydrophilic proteins which are called late embryogenesis abundant (LEA) polypeptides (Galau & Hughes 1987; Galau et al. 1987, 1991), although some are synthesized from about the mid-point of seed development, have long been implicated in cellular protection during both seed desiccation and water stress (see Skriver & Mundy 1990; Bray 1993; Chandler et al. 1993; Dure 1993, for reviews). In mature cotton embryos, where they were first described, they make up about 2% of the total soluble protein and about 30% of the non-storage protein. Within the cells of the embryo, the LEA proteins are uniformly localized throughout the cytoplasm (Roberts et al. 1993). LEA proteins or *lea* transcripts have now been reported in the mature embryos of many species of monocots, dicots and gymnosperms. These proteins may act as desiccation protectants, and transcription of *lea* genes can be elicited by desiccation of cotton embryos at the early stage of maturation (Galau et al. 1991). Proteins related to some of the LEAs, e.g., dehydrins in barley, pea and maize, and RAB proteins in rice seedlings, can be induced by water stress and in response to exogenous ABA (Ingrams & Bartels 1996)

LEA proteins have physical properties consistent with a role in desiccation tolerance, e.g., they are extremely hydrophilic and resistant to denaturation (Close et al. 1989; Dure et al. 1989). These proteins may solvate cellular components such as other proteins and membranes, and thus protect them from drying-induced damage or disruption by providing a surrogate water film. Other LEA proteins may form amphiphilic helices that sequester ions that are concentrated during maturation drying (Baker et al. 1988; Roberts et al. 1993).

A set of LEA proteins appears in developing barley and maize embryos about the time that tolerance of desiccation is acquired. A small subset of these proteins are induced when barley embryos at the intolerant stage of their development are cultured in ABA (Bartels et al. 1988; Bochicchio et al. 1991), and a causal relationship between ABA and *lea* gene expression *in vivo* has been suggested. Evidence for and against this relationship exists in the literature (see Oliver & Bewley 1997, for review). It is possible that LEA protein production is regulated by different

mechanisms in different seeds, but it is by no means clear that ABA plays a direct role in the induction and maintenance of expression of LEA proteins in seeds, or that these proteins are integrally involved in the imposition of desiccation tolerance.

In the maturing seeds of several species, concentrations of certain sugars and oligosaccharides increase at the onset of desiccation tolerance (Amuti & Pollard 1977; Koster & Leopold 1988; LePrince et al. 1990; Chen & Burris 1990; Blackman et al. 1992), and thus may be components of a protective mechanism. The disaccharide sucrose and the oligosaccharides raffinose and stachyose increase late in development; during earlier developmental stages, the monosaccharides glucose, mannose, fructose and galactose are more prominent. There is an increase in sucrose, raffinose, and particularly stachyose in soybean embryos induced to become desiccation-tolerant by slow drying (Blackman et al. 1992) but not in those maintained in the intolerant state.

The cytoplasm of dry seeds exists in a glassy (vitrified) state in which chemical reactions requiring molecular diffusion are greatly reduced. This prevents damaging interactions between cell components. Denaturation of enzymes is retarded or averted because they are held in their stable, folded state. Vitrification also prevents crystallization of solutes in the cytoplasm (Burke 1986; Leopold et al. 1992). The importance of sugars and oligosaccharides in tolerance of drying is related to their role in the formation of this glassy state and in the protection of membranes and vital proteins.

How does the mechanism of tolerance in the vegetative tissues of modified desiccation tolerant plants compare to what we know of the seed mechanism?

Drying and ABA

Attached and detached leaves of *Craterostigma plantagineum* can survive desiccation if dried to 15% of fresh weight over 24 to 48 h (Bartels et al. 1990). Callus derived from the leaf tissue of this plant is not inherently desiccation-tolerant but becomes so if treated for four days with ABA before drying (Bartels et al. 1990). ABA increases six- to seven-fold in leaf tissues during slow drying. Many new proteins are synthesized in both callus and leaf tissue during drying and when ABA is applied to non-stressed tissues (Bartels et al. 1990). By using differential screening, cDNAs corresponding to transcripts expressed only in desiccation-tolerant tissues were isolated and characterized (Bartels et al. 1990; Piatkowski et al. 1990; Bartels et al. 1992; 1993). The majority of the cDNAs represent transcripts that also increase greatly in abundance following ABA treatment; some are expressed within the first 30 min of drying and others appear later (Bartels et al. 1990). The differing kinetics of expression during drying and the requirement for ABA for the induction of desiccation tolerance in callus have led to the hypothesis that ABA co-ordinates the activation of genes by moderate drying, leading to cellular tolerance of extreme drying (Bartels et al. 1993). However, recent evidence that a desiccation-induced homeodomain-leucine zipper protein, expressed early in the drying process, is not induced by ABA raises the possibility that other signaling pathways are involved in desiccation tolerance in *Craterostigma* (Frank et al. 1998). This would be consistent with the conclusion that other stress-related gene expression responses utilize multiple signaling pathways (Ishitani et al. 1997)

A gene, CDT-1, has recently been identified that apparently acts downstream of ABA to activate a pathway that renders *Craterostigma* callus desiccation-tolerant. Over-expression of CDT-1 in callus results in desiccation tolerance without the addition of ABA, apparently by the induction of a set of genes that are normally induced by ABA in wild-type callus (Furini et al. 1997). This regulatory gene has some unusual properties, i.e., structural features similar to mammalian retrotransposons. At present it is unclear if its activity is mediated by a small polypeptide or via a transcribed RNA activator.

The involvement of ABA in the initial induction of desiccation tolerance during a drying event may not be universal in the angiosperms. *Sporobolus stapfianus* can survive desiccation at rates that are similar to those that *Craterostigma* can survive. However, detached leaves of *Sporobolus* do not survive equilibration to an atmosphere of below 92% relative humidity, which is similar to the limits of tolerance in the leaves of normal, non-tolerant crop plants (Gaff & Ellis 1974). For detached leaves to exhibit desiccation tolerance, their relative water content has to be 61% or lower before they are removed from the parent plant. At this level of water stress, endogenous ABA has just started to increase in the leaves; ABA content peaks at a much lower water content, suggesting an ABA-independent mechanism. In addition, the application of ABA does not appreciably alter the extent of tolerance exhibited by detached leaves (Gaff & Loveys 1994). Protein profiles indicate that novel proteins are synthesized during different stages of drying in *Sporobolus* (Gaff

et al. 1993; Kuang et al. 1995). The first set of novel proteins is synthesized between 85% and 50% RWC, prior to the elevation in internal ABA levels. A second set is synthesized at around 37% RWC. Thus it appears that ABA induction of gene expression does play an important role in the establishment of desiccation tolerance but only later in the drying process for *Sporobolus stapfianus* (Blomstedt et al. 1998). Analysis of individual genes that are induced by desiccation of *Sporobolus* leaves confirms the presence of both ABA-independent and ABA-dependent components of desiccation tolerance in this grass (Blomstedt et al. 1998).

In the pteridophyte *Polypodium virginianum*, ABA does not increase in leaf tissues during drying. Rather, it decreases, especially after fresh weight has declined by 20% (Reynolds & Bewley 1993a). Nevertheless, application of ABA to the fronds results in the synthesis of proteins similar to those seen during desiccation, and pretreatment of *Polypodium* fronds with ABA allows them to survive rapid desiccation (Reynolds & Bewley 1993a,b). How ABA is involved in this response remains an enigma.

At least one bryophyte exhibits a mechanism of tolerance that appears similar to that of the modified desiccation-tolerant angiosperms and pteridophytes, and ABA may be involved. Protonema of *Funaria hygrometrica* Hedw. grown in culture tolerate slow desiccation but die if water loss is rapid (Werner et al. 1991). ABA increases in the protonema during drying, and they can survive rapid desiccation if they have previously been dried slowly and then rehydrated. The application of ABA to protonema also enables them to survive rapid drying (Werner et al. 1991). Bopp & Werner (1993) reported that ABA exerts its influence through the synthesis of specific proteins that are synthesized during drying and indicated that some of these resemble dehydrins. Thus it is possible that some tolerant bryophytes have independently evolved mechanisms for desiccation tolerance similar to those in the tracheophytes. It is interesting to note that ABA has not been found in *T. ruralis ruralis* as discussed above.

Proteins

Several of the cDNA clones isolated from *Craterostigma plantagineum* (Bartels et al. 1993) and *Sporobolus stapfianus* (Blomstedt et al. 1998) are related to LEAs and dehydrins. Other genes whose transcription is initiated in response to drying have been isolated from both plants but their roles in desiccation tolerance (with the exception of CDT-1 described above) remain unclear (Bockel et al. 1998; Oliver & Bewley 1997; Blomstedt et al. 1998). Some appear to be involved in the response of the chloroplast to desiccation, e.g., the *Craterostigma* dsps 21, 22, and 34 polypeptides. All are localized in the chloroplasts of leaf cells; dsps 21 and 22 are localized in the stroma. All are strongly inducible by ABA in leaves and callus; dsp 22 appears to be regulated also by light. Dsp 22 is closely related to plant early light-inducible genes (Elip) and to a carotene biosynthesis-related gene from a green alga (Bartels et al. 1992). Dsp 34 is associated with thylakoids and is only present in dried leaf tissue and not in callus, even if the latter is ABA-treated prior to drying. A gene that is not induced by ABA but is induced during desiccation and again upon rehydration of *Sporobolus*, SsRab2, is a small GTP-binding protein that is associated with vesicular trafficking in other systems. This process has importance in maintaining membrane integrity and hence may be of importance to both cellular protection and repair (O'Mahony & Oliver 1998).

Slow drying of *Polypodium* fronds also induces the synthesis of novel proteins, most noticeably a doublet with a low molecular mass of 19–29 kD (Reynolds & Bewley 1993a). The same novel proteins are synthesized during rapid drying; since fronds do not survive this, the newly synthesized proteins per se cannot account for the desiccation tolerance of *Polypodium*. This fern also synthesizes dehydrin-like proteins in response to drying. *Polypodium* dehydrins are larger than those generally found in angiosperms and are closer in mass to those of *T. ruralis* (Bewley et al. 1993).

Rehydration of slow-dried fronds of *P. virginianum* results in the rapid disappearance of proteins synthesized during drying, and they are no longer present after 6 h of rehydration. Several proteins that decline in amount during desiccation, including four thylakoid proteins (Reynolds 1992), increase again after 24 h of rehydration to amounts present in the undesiccated frond. Rehydration also results in the synthesis of specific proteins. Within the first 3 h of rehydration, there are 18 novel polypeptides synthesized that are not synthesized during desiccation. The synthesis of these proteins is also transient since it ceases after 6 h of rehydration; however, a new set of at least 22 new proteins is synthesized later (up to 24 h) after rehydration (Reynolds & Bewley 1993a). This is somewhat analogous to the synthesis of rehydrins in *T. ruralis* and indicates that there maybe a repair-based com-

ponent in the mechanism of tolerance exhibited by *Polypodium*.

Sugars

Desiccation of *Craterostigma* also induces a major change in carbohydrate metabolism during water loss that may be directly related to desiccation tolerance. Under normal, hydrated conditions, leaves of *Craterostigma* contain the unusual carbohydrate 2-octulose, which accumulates to nearly 50% of dry weight (Bianchi et al. 1991a, 1992). During drying, this sugar is rapidly converted into sucrose. Such increases in sucrose and other sugars or sugar derivatives occur in several desiccation-tolerant species: e.g., sucrose and trehalose in *Myrothamnus flabellifolia* Welw. (Bianchi et al. 1993; Drennan et al. 1993), a-trehalose in *Selaginella lepidophylla* (Gaff 1989); sucrose in *Boea hygroscopica* (F.) Meull. (Kaiser et al. 1985; Bianchi et al. 1991b) *Ramonda nathaliae* Panc. & Petrov., *Ramonda myconi* (l.) Reichenb. and *Haberlea rhodopensis* Friv. (Muller et al. 1997); and cardiomanol, a novel glucoside in *Cardiomanes reniforme* (Forst.) C. Presl. (also named *Trichomanes reniforme* Forst.) and the grasses *Sporobolus staphianus* and *S. festivas* Hoscht (Kaiser et al. 1985). These observations, along with a body of work demonstrating that sugars stabilize membranes during drying (see Crowe et al. 1992, for review), support the idea that the accumulation of sugars during drying is an integral part of vegetative (as well as propagative) desiccation tolerance.

Poikilochloryphyllous desiccation-tolerant plants

Poikilochlorophyllous desiccation-tolerant plants occur only among plants that are modified desiccation-tolerant and appear to be restricted to monocots (Gaff 1977, 1989; Bewley & Krochko 1982).). Poikilochlorophylly is currently known in eight genera of four families (Gaff 1989; unpublished data of Tuba et al.). All occupy the almost soil-less, tropical rocky outcrops known as inselbergs (Barthlott et al. 1993). Only a few species of poikilochlorophyllous desiccation-tolerant plants have been investigated so far for insights into the mechanism of desiccation tolerance and breakdown of the photosynthetic apparatus. The main experimental species have been three African shrubs in the Velloziaceae, *Xerophyta scabrida* (Tuba et al. 1993a), *X. villosa* (Halam & Luff 1980) and *X. viscosa* (Sherwin & Farrant 1998), and a member of the Liliceae, *Borya nitida* Labill., from Western Australia (Gaff et al. 1976; Hetherington & Smillie 1982). Ultrastructural analysis of dry, viable leaves of *Borya nitida* revealed that the integrity of most of the cell structure is maintained during drying with the exception of plastids (Gaff et al. 1976). Starch grains were also lost, indicating a build-up of sucrose during drying, as in other modified tolerant angiosperms. Later work indicated the involvement of ABA in the response to desiccation in this plant (Gaff & Loveys 1994).

In a more extensive study, Tuba et al. (1993a,b) followed the reconstitution of plastids and the resynthesis of the photosynthetic apparatus following rehydration of dried leaves of *X. scabrida*. In dried leaves, the thylakoid system within the chloroplasts had been completely replaced by small groups of plastoglobuli and osmophilic, stretched lipid material. Similar structures have been reported for *X. villosa* (Halam & Luff 1980) and *X. viscosa* (Sherwin & Farrant 1996). Tuba et al. (1993b) termed these former chloroplasts ‘desiccoplasts’ to distinguish them from other chloroplast-derived structures seen in senescing leaves. The elongated osmophilic structures appear to occupy the positions previously occupied by the thylakoids. Ten to twelve hours following the reintroduction of water, when full turgor and the maximum leaf water content are reached, the synthesis of chlorophylls and carotenoids along with the reassembly of the thylakoids is initiated. The stacking of two primary thylakoids to form a granal stack is an early step in the reconstitution of the chloroplasts. The newly formed thylakoids become increasingly functional and by 72 h the chloroplasts are normal and full photosynthetic capacity is restored (Tuba et al. 1993b , 1994). Respiration recovers rapidly upon rehydration of *X. scabrida* and is fully operational prior to the leaves reaching full turgor.

Tuba et al. (1996, 1997) further examined the physiological changes associated with desiccation in *X. scabrida*. A reduction in the rate of photosynthesis during drying, as measured by net CO_2 assimilation, is associated with decreases in chlorophyll a+b content , photochemistry and stomatal conductance. After CO_2 assimilation ceases, the disorganization of the photosynthetic system begins, and the chlorophyll is degraded, as reflected in the chlorophyll/carotenoid ratio (Tuba et al. 1996). Respiration is much less affected by desiccation than photosynthesis and is detectable until near the end of the drying period. Res-

piration is linearly related to tissue water content on a fresh weight basis (Tuba et al. 1997), and *X. scabrida* maintains respiration for a significantly longer period during laboratory drying than some homiochlorophyllous desiccation-tolerant angiosperms. This prolonged 'desiccation respiration' may provide energy for controlled breakdown of the photosynthetic pigments and for disassembly of the thylakoid structure required for formation of desiccoplasts (Tuba et al. 1997) demonstrated that desiccation respiration is a consequence of the slow drying rates of *X. scabrida* compared to those of homiochlorophyllous desiccation-tolerant plants. Slow drying is apparently due to an extensive reduction in the ratio of leaf area to leaf weight in *X. scabrida* during drying. Changes in ratio of leaf surface area to leaf biomass during desiccation may have an essential role in desiccation tolerance in the poikilochlorophyllous monocots (Tuba et al. 1994).

Under laboratory conditions *X. scabrida* leaves took 16 days to dry out completely (Tuba et al. 1997), six times longer than reported for other poikilochlorophyllous desiccation-tolerant plants desiccating in their natural habitats (Gaff 1977). Nevertheless, this extended drying period was still not enough to bring about the complete loss of chlorophyll that occurs in *X. scabrida* in nature. In natural habitats, the desiccation period may therefore be longer than 16 days.

The main function of pokilochlorophylly is probably to limit photo-oxidative damage that can result from the uncoupling of carbon fixation from the electron transport pathway under cellular water deficits (Smirnoff 1993). This advantage, together with the possible benefits from not having to maintain the photosynthetic apparatus intact through long periods of desiccation, presumably outweighs the disadvantage of slow recovery (Tuba et al. 1998). If so, the reversible loss of plastid structure during desiccation may be thought of as an ordered deconstruction and reconstruction process, added to cellular protection mechanisms of vegetative desiccation tolerance. Poikilochlorophylly may thus be the most highly derived form of desiccation tolerance in plants.

Current evolutionary inferences

Although the mechanisms of desiccation-tolerant plants have been studied in only a few species so far, and some key points in their phylogeny are unclear, the recent, synthetic phylogenetic analyses summarized in Figures 1 and 2 have allowed us to construct a relatively strong and testable hypothesis concerning the evolutionary history of this important trait in plants. Because of its widespread occurrence in the bryophytes, the basal-most living clades of land plants, it is likely that vegetative desiccation tolerance was primitively present in the land plants. It was then lost in the evolution of tracheophytes and subsequently re-evolved multiple times in separate lineages. We postulate that the initial evolution of vegetative desiccation tolerance was a crucial step required for the colonization of the land by primitive plants from fresh water (Mishler & Churchill 1985). The primitive mechanism of tolerance exhibited by the first plants probably involved a constitutive level of cellular protection coupled with an efficient and active repair process, similar to what we have described for modern-day desiccation-tolerant bryophytes.

However, this desiccation tolerance came at a cost, because metabolic rates are low in tolerant plants as compared to plants that do not maintain costly mechanisms for tolerance. As plants evolved to fill the various niches available to them on dry land, loss of tolerance was favored because of the internalization of water relations as the vascular plants became larger and more complex. Vegetative desiccation tolerance was lost in preference for the advantages afforded to plants by increased growth rates, structural and morphological complexity, and mechanisms that conserve water within the plant while maintaining efficient carbon fixation. Genes that had evolved for cellular protection and repair were, in all likelihood, recruited for different but related processes such as the response to water stress and, more important, the desiccation tolerance of reproductive propagules. The mechanism of desiccation tolerance exhibited in seeds thus may have evolved secondarily from more primitive forms of vegetative desiccation tolerance.

Once established in seeds, the developmentally induced cellular protection system became available for induction in vegetative tissues by environmental cues that are related to drying. We hypothesize that a more recent, modified vegetative desiccation tolerance mechanism evolved from that programmed into seed development as certain species spread into very arid environments. Within the angiosperms, at least eight independent cases of re-evolution of desiccation tolerance occurred. Most recently, in response to the rigors of being dry in marginal habitats with high irradiance, certain desiccation-tolerant monocots evolved the strategy of poikilochlorophylly.

Each time the general phenotype was re-evolved, the time scale of desiccation, rehydration, and responsiveness were different. The desiccation-tolerant plants that belong to the less complex clades and that exhibit the ability to withstand rapid desiccation are adapted to habitats where cycles of wetting and drying generally occur within a few hours. Homiochlorophyllous modified desiccation-tolerant plants are adapted to longer cycles, extending from many hours to days or weeks, and the poikilochlorophyllous modified desiccation-tolerant plants to cylcles lasting from weeks to months. Of course there is variation within each category, the categories overlap in their ecological adaptation (Tuba et al. 1998), and species from two or more categories may coexist in one habitat (Ibisch et al. 1995).

This hypothesis does not yet explain all the known facts about the comparative mechanisms of desiccation tolerance in plants. Pteridophytes apparently have a mechanism that is intermediate between the more primitive mechanism exhibited by *Tortula ruralis* and the modified tolerant mechanisms of angiosperms. Like the higher plants tested but unlike almost all of the bryophytes, the pteridophytes synthesize novel proteins during desiccation, apparently under the control of ABA. However, unlike angiosperms but like bryophytes, the pteridophytes synthesize rehydrins. The mechanism of desiccation tolerance in pteridophytes may represent a separate evolution of vegetative desiccation tolerance that was more directly derived from the primitive form than is the case in other modified tolerant plants. This may also be true of some bryophytes, such as *Funaria*, which have a mechanism that relies on the induction of a cellular protection system. Further characterization and comparisons to the angiosperm examples have to be conducted before this becomes clear.

Finally, the study of vegetative desiccation tolerance in plants is still in its infancy and much work utilizing a variety of plants as model systems needs to be accomplished. We hope that the synthetic hypothesis we have generated will help direct continued study to new species selected to yield the most valuable insights into the evolution of the important and amazing trait of desiccation tolerance.

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